NICHOLAS C. PRICE
LEWIS STEVENS

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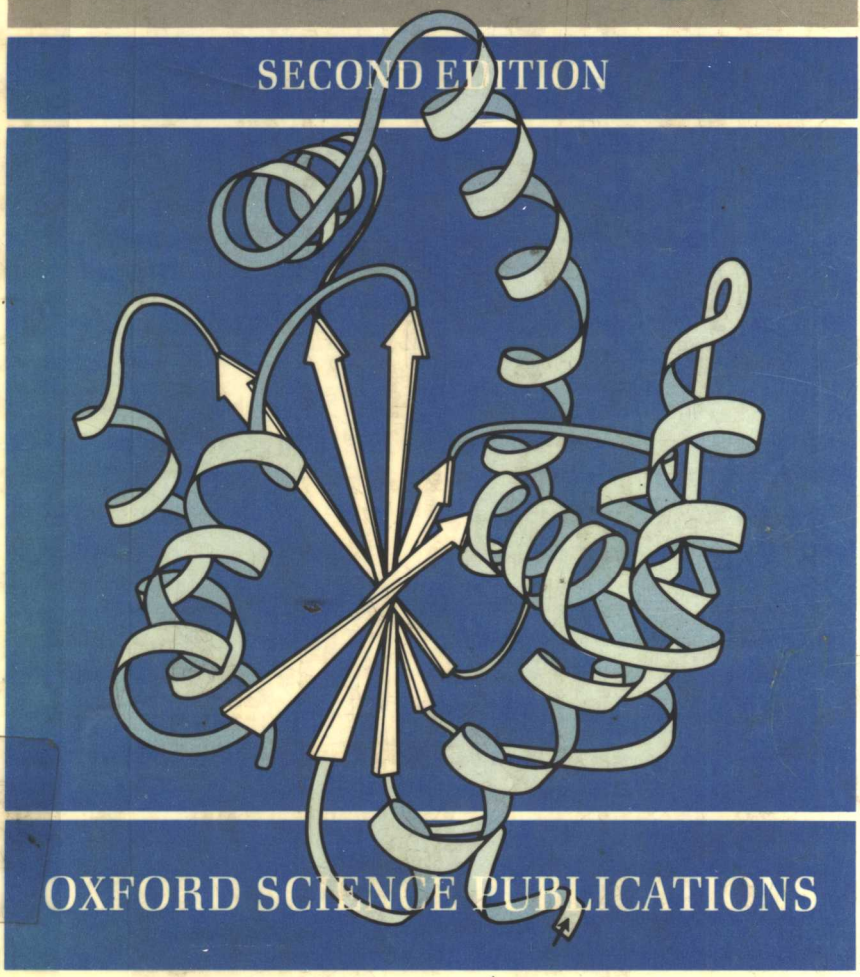
SECOND EDITION



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Fundamentals of Enzymology

SECOND EDITION

BY

NICHOLAS C. PRICE
Reader in Biochemistry, University of Stirling
AND
LEWIS STEVENS
Senior Lecturer in Biochemistry, University of Stirling

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Quantity	SI unit
	since the definition of standard states is then less cumbersome, being under a pressure of 1 atm rather than 101.325 kPa. (1 atm = 760 mm Hg = 101.325 kPa)
Energy	*joule* (J) ($1\ m^2\ kg\ s^{-2}$). One calorie = 4.18 J. The gas constant, $R = 8.31\ J\ K^{-1}\ mol^{-1}$ (1 electron volt = $96.5\ kJ\ mol^{-1}$)
Electric charge	*coulomb* (C) (1 ampere second). Faraday constant = $96\,500\ C\ mol^{-1}$
Frequency	*hertz* (Hz) ($1\ s^{-1}$)
Viscosity	the units are $kg\ m^{-1}\ s^{-1}$ (Note that 1 centipoise = $10^{-3}\ kg\ m^{-1}\ s^{-1}$).

The main implications of these units for biochemists are as follows.

(i) Temperatures are quoted in kelvin (or absolute). Thus 25 °C is 298.15 K (in practice, this is given as 298 K).
(ii) Enthalpy, internal energy, and free-energy changes are given in $J\ mol^{-1}$.
(iii) Entropies are quoted in $J\ K^{-1}\ mol^{-1}$.
(iv) Molar concentrations are given in terms of $mol\ dm^{-3}$ rather than M.
(v) Enzyme activities are quoted in terms of katals (the amount of enzyme catalysing the transformation of 1 mol substrate per second). Specific activities are quoted in terms of katal kg^{-1}.
(vi) The curie (Ci) is redundant as a unit of radioactivity; 1 Ci = 3.7×10^{10} disintegrations s^{-1}. (The unit disintegrations s^{-1} is given the name *becquerel* (Bq)).

For fuller discussions of SI units the following may be consulted:

Quantities, units, and symbols (2nd edn). The Royal Society, London (1975).
Physicochemical quantities and units (2nd edn). M. L. McGlashan. Royal Institute of Chemistry, London (1971).

List of abbreviations

The following standard abbreviations, which are in common use in biochemistry, are used in the text. Any others are defined where used.

A	absorbance
ACP	acyl-carrier protein
ADP	adenosine 5′-pyrophosphate (adenosine 5′-diphosphate)
AMP	adenosine 5′-phosphate (adenosine 5′-monophosphate)
ATP	adenosine 5′-triphosphate
cAMP	3′:5′ cyclic AMP
cDNA	complementary DNA
CoA	coenzyme A
CTP	cytidine 5′-triphosphate
dATP	2′-deoxyadenosine 5′-triphosphate
dGDP	2′-deoxyguanosine 5′-pyrophosphate (2′-deoxyguanosine 5′-diphosphate)
dGTP	2′-deoxyguanosine 5′-triphosphate
DNA	deoxyribonucleic acid
dTMP	thymidine 5′-phosphate (thymidine 5′-monophosphate or 2′-deoxyribosylthymine 5′-monophosphate)
dTTP	thymidine 5′-triphosphate (2′-deoxyribosylthymine 5′-triphosphate).
dUMP	2′-deoxyuridine 5′-phosphate (2′-deoxyuridine 5′-monophosphate)
EDTA	ethylenediaminetetra-acetate
FAD	flavin-adenine dinucleotide (oxidized form)
$FADH_2$	flavin-adenine dinucleotide (reduced form)
g	gravitational field, unit of ($9.81\ m\ s^{-2}$)
GDP	guanosine 5′-pyrophosphate (guanosine 5′-diphosphate)
GTP	guanosine 5′-triphosphate
h.p.l.c.	high-performance liquid chromatography
i.r.	infrared
ITP	inosine 5′-triphosphate
mRNA	messenger RNA
M_r	relative molecular mass
NAD^+	nicotinamide-adenine dinucleotide (oxidized form)
NADH	nicotinamide-adenine dinucleotide (reduced form)
$NADP^+$	nicotinamide-adenine dinucleotide phosphate (oxidized form)
NADPH	nicotinamide-adenine dinucleotide phosphate (reduced form)
NMN	nicotinamide mononucleotide
n.m.r.	nuclear magnetic resonance
RNA	ribonucleic acid
r.p.m.	revolutions per minute

rRNA	ribosomal ribonucleic acid
tris	2-amino-2-hydroxymethyl-propane-1, 3-diol (tris(hydroxymethyl) methylamine)
tRNA	transfer RNA
UDP	uridine 5′-pyrophosphate (uridine 5′-diphosphate)
UMP	uridine 5′-phosphate (uridine 5′-monophosphate)
UTP	uridine 5′-triphosphate
u.v.	ultraviolet

Nucleotide sequences

A sequence of nucleotides can be written as, e.g., A C G C U C where each letter signifies a nucleotide.* The convention is to write the order so that the phosphodiester link runs 3′ to 5′ from left to right. A nucleoside 5′-phosphate is written as pX, and a 3′-phosphate as Xp. The sequence above could also be written as ApCpGpCpUpC. To indicate that there is a phosphate, pyrophosphate or triphosphate group at the 5′-end, we would write

pA C G C U C	5′-phosphate
ppA C G C U C	5′-pyrophosphate
pppA C G C U C	5′-triphosphate

* The nucleotide is taken to be a ribonucleotide unit, unless the context makes it clear that it is a deoxyribonucleotide (e.g. in DNA). In a DNA sequence, the letter T refers to a thymidine phosphate (2′-deoxyribosylthymine phosphate) unit.

Contents

A note on units

In this book we have tried, wherever possible, to use SI units. These are based on the metre-kilogram-second system of measurement. Multiples of the basic units by powers of 10 are as follows.

Prefix		Abbreviation	Prefix		Abbreviation
$10(10^1)$	deca	da	10^{-1}	deci	d
10^2	hecto	h	10^{-2}	centi	c
10^3	kilo	k	10^{-3}	milli	m
10^6	mega	M	10^{-6}	micro	μ
10^9	giga	G	10^{-9}	nano	n
10^{12}	tera	T	10^{-12}	pico	p

We do not use compound prefixes, thus 10^{-9} metre (m) = 1 nm, not 1 mμm. SI units for the various physical quantities mentioned in this book are listed below.

Quantity	SI unit
Time	*second* (s).
Length	*metre* (m). (Supplementary units retained for convenience are dm, cm)
Mass	*kilogram* (kg). Note that multiples are based on 1 gram (g), i.e. mg, μg rather than μkg, nkg (see above compound prefix rule)
Volume (given in units of length cubed)	*cubic metre* (m^3). For convenience the litre ($1l = 1\ dm^3$) and millilitre ($1\ ml = 1\ cm^3$) are retained
Amount of substance	*mole* (mol). This quantity contains 1 Avagadro number of basic units (e.g. electrons, atoms, or molecules)
Concentration	*mol* dm^{-3} used instead of molar (M) *mol* kg^{-1} used instead of molal (m)
Temperature	*kelvin* (*K*) note 0 °C = 273.15 K
Force	*newton* (N) ($1\ m\ kg\ s^{-2}$)
Pressure	*pascal* (Pa) ($1\ Nm^{-2}$). In this text, however, we have retained the atmosphere unit (atm)

1
Introduction

1.1 Aims of the book

In this book we have tried to give a broad account of enzymology and have aimed to put current knowledge into perspective. Studies of enzymes have as their ultimate goal an understanding of the crucial role that these catalysts play in the metabolic processes of living organisms. Because of the complexity of such processes, it is at least necessary to gain an insight into the properties of enzymes in simpler systems, i.e. as isolated entities studied in the test tube or spectrophotometer cuvette. The chapters in the book follow a progression from the properties of isolated enzymes to the behaviour of enzymes in increasingly complex systems, leading up to the cell. We have included some discussion of the importance of enzymes in medicine and industry to emphasize that enzymology is not a purely academic subject but has increasingly wide applications.

1.2 Historical aspects

Enzymes are catalysts (i.e. they speed up the rates of reactions without themselves undergoing any permanent change). Each reaction taking place in the cell is catalysed by its own particular enzyme so that in a given cell there are a large number of enzymes. It is difficult to make a precise estimate of the number of different enzymes in each cell but it seems that a bacterial cell such as *Escherichia coli* makes about 3000 different proteins and a higher eukaryote cell about 50 000 of which the majority are enzymes. In the absence of enzymes many of these reactions would not occur even over a time period of years and life as we know it could not exist.

The word *enzyme* is derived from the Greek meaning 'in yeast' and was first used by Kühne in 1878.* At the time it was used to distinguish between what were referred to as 'organized ferments' (meaning whole microorganisms) and 'unorganized ferments' (meaning extracts or secretions from whole organisms). The term *enzyme* was thus intended to emphasize that catalytic activity was 'in yeast', i.e. a manifestation of an extract or secretion rather than of the whole organism. Although the term specified yeast, it was to be used for all 'unorganized ferments'. Of course, the catalytic activity of enzymes in microorganisms has been utilized by man for many thousands of years in processes such as fermentation and cheese-making but this was very much purely a practical use. It was only when it was shown that enzyme activity could be

* It was pointed out by Fruton[1] that the term was used as far back as the twelfth century by the Armenian philosopher, Theorianus—although obviously not in a biochemical context.

expressed without the need for an intact cellular structure that the study of enzymes could proceed along the paths already established in the study of chemistry. In this respect Büchner's demonstration (1897) that filtrates of yeast extracts could catalyse fermentation was highly significant.

Emil Fischer in 1894 had performed some classical studies on carbohydrate metabolizing enzymes in which he demonstrated the specificity shown by an *enzyme* for its *substrate* (the molecule acted on by the enzyme). On the basis of his experiments, Fischer proposed the 'lock and key' hypothesis to describe this interaction and for many years this proved to be a fruitful way to picture the binding of enzyme to substrate (Fig. 1.1)

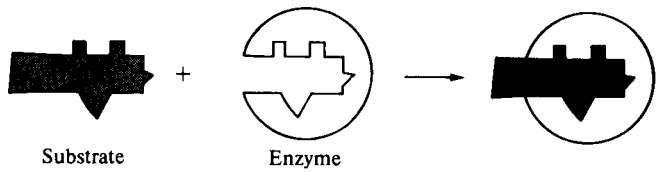

FIG. 1.1. Fischer's 'lock and key' hypothesis to explain enzyme specificity.

At this time (1890s) the chemical nature of enzymes was not clear. In fact this point was only established many years later after a number of enzymes had been crystallized and been shown to consist entirely of protein (i.e. made up of amino acids linked by amide bonds, see Chapter 3, Section 3.3). The first enzyme to be crystallized (in 1926 by Sumner) was urease, which catalyses the hydrolysis of urea to yield carbon dioxide and ammonia. Actually, it is now known that if Sumner had had more sensitive methods of analysis he would have found that the preparation contained a small amount of nickel (approximately 0.1 per cent by weight) which is essential for catalysis.[2] Perhaps in retrospect it was fortunate that he did not have such sensitive methods, since otherwise the nature of enzymes might not have been settled for many more years.

The development of the ultracentrifuge by Svedberg (also in the 1920s) allowed very high centrifugal fields capable of sedimenting macromolecules* to be generated. These studies showed that proteins in solution generally consist of homogeneous molecules of definite M_r (in the case of enzymes the M_r values range between about 10^4 and 10^7) rather than of colloidal suspensions. The description of enzyme structures in precise chemical terms was then a realistic possibility: this was first achieved in 1960 when the amino-acid sequence of ribonuclease[3] (an enzyme catalysing the hydrolysis of ribonucleic acid) was deduced. In 1965 the three-dimensional structure of lysozyme (an enzyme cleaving certain bacterial cell walls) was deduced by the technique of X-ray crystallography (see Chapter 3, Section 3.5.1), and for the first time a mechanism of action could be postulated in precise structural terms. We now know the

* The term macromolecule is generally reserved for molecules of relative molecular mass (M_r) > 10000

amino-acid sequence and three-dimensional structure of many enzymes and generalizations and comparisons between them can be undertaken.

During the late 1950s and the 1960s a number of observations were made that suggested that enzymes show considerable flexibility. In 1958 Koshland[4] proposed the 'induced fit' theory to account for the catalytic power and specificity shown by enzymes (see Chapter 5, Section 5.6). It also became clear that the catalytic activity of certain enzymes could respond to changes in physiological conditions. Monod and his colleagues[5,6] proposed their 'allosteric model' to explain in a quantitative way how the activity of certain enzymes can be regulated by the binding of small molecules (*effectors*) and this provided a basis for understanding many features of the control of enzymes in the cell. An important feature of models for allosteric enzymes in general is that they postulate that the binding of effectors to the enzymes induces structural changes in the enzymes (Chapter 6, Section 6.2.2).

The first chemical synthesis of an enzyme (ribonuclease) from amino-acid precursors was reported in 1969.[7] Although this represented a considerable achievement, it should be noted that both the chemical purity and catalytic activity of the preparation were rather low. The chemical synthesis can, perhaps, be said to represent the final proof that enzymes are no different qualitatively from other non-biological catalysts.

The application of recombinant DNA techniques to the study of enzymes has produced some remarkable new insights (see Chapter 3, Section 3.4.1 and Chapter 5, Sections 5.4.5 and 5.5). It has proved possible to alter catalytic activity and specificity in a rational manner by introducing mutations at defined positions using site-directed mutagenesis. This has helped in understanding the mechanism of enzyme action and has also opened the prospect of designing enzymes with specific required properties.[33] For example, the specificity of lactate dehydrogenase has been changed to that of malate dehydrogenase by introducing three particular mutations at the active site (see Chapter 5, Section 5.5.4.5).

Recently it has become evident that catalytic activity can be shown to a limited extent by biological molecules other than the 'classical' enzymes. In the process of enzyme catalysis the substrates are converted via a high-energy transition state to the eventual products (see Chapter 5, Fig. 5.1). A number of reports have shown that antibodies raised to stable analogues of the transition states of hydrolytic reactions (see Chapter 5, Section 5.3.4) can act as moderately effective catalysts for those reactions.[30–32] Fragments of RNA can also act as catalysts for reactions involving hydrolysis of RNA.[8,9] The observation of these 'ribozymes' has important implications for theories concerning the evolution of catalytic function.[10]

For further details on the history of enzymology see references 11 and 12.

1.3 Remarkable properties of enzymes as catalysts

Enzymes display a number of remarkable properties when compared with other types of catalyst. The three most important are their high catalytic power, their

specificity, and the extent to which their catalytic activity can be regulated by a variety of naturally occurring compounds. These three properties will be illustrated below.

1.3.1 Catalytic power

Enzymes may increase the rate of a reaction by as much as 10^{14}-fold. There are not many examples where a direct comparison can be made between the rates of an enzyme-catalysed reaction and the reaction occurring under similar conditions of temperature, pH, etc. but in the absence of enzyme. This is because in the absence of an enzyme the rates may be too low to be measured easily. Where the comparison has been made, very high rate enhancements have been found,[13] e.g. hexokinase $> 10^{10}$, phosphorylase $> 3 \times 10^{11}$, alcohol dehydrogenase $> 2 \times 10^{8}$, and creatine kinase $> 10^{4}$. In other instances, where enzymatic and non-enzymatic catalysts are compared, the former catalyse much higher rates and in some cases do so at significantly lower temperatures (Table 1.1). The optimum conditions for enzyme catalysis are almost invariably moderate temperatures, and pHs that are not extreme. The contrast between an enzyme-catalysed reaction and that catalysed by a non-enzymatic catalyst is well illustrated by the process of nitrogen fixation (i.e. reduction of N_2 to ammonia). Nitrogenase* catalyses this reaction at temperatures around 300 K and at neutral pH. The enzyme is a complex system comprising two dissociating protein components, one of which contains iron and the other iron and molybdenum.[15] Several molecules of ATP are hydrolysed during the reduction, although the exact stoichiometry is still uncertain. By contrast, in the industrial synthesis of ammonia from nitrogen and hydrogen the conditions used are as follows:

TABLE 1.1
Examples of the catalytic power of enzymes

Substrate	Catalyst	Temperature (K)	Rate constant k $(\text{mol dm}^{-3})^{-1}\,\text{s}^{-1}$
Amide (hydrolysis)			
benzamide	H^+	325	2.4×10^{-6}
benzamide	OH^-	326	8.5×10^{-6}
benzoyl-L-tyrosinamide	α-chymotrypsin	298	14.9
Urea	H^+	335	7.4×10^{-7}
(hydrolysis)	urease	294	5.0×10^{6}
$2H_2O_2 \rightarrow 2H_2O + O_2$	Fe^{2+}	295	56
	catalase	295	3.5×10^{7}

Data taken from reference 14.

* Only certain prokaryotes can carry out nitrogen fixation and they may be either symbionts or non-symbionts. In terrestrial ecosystems, symbiotic fixation appears to exceed non-symbiotic and the most outstanding example of symbiotic fixation is that between bacteria of the genus *Rhizobium* and the roots of leguminous plants.

temperatures between 700 and 900 K, pressures between 100 and 900 atmospheres, and the presence of an iron catalyst, often promoted by traces of oxides of other metals.[16] The basis of catalytic power is discussed in Chapter 5, Section 5.3.

1.3.2 Specificity

Most enzymes are highly specific both in the nature of the substrate(s) they utilize and also in the reaction they catalyze. The range of specificity varies between enzymes. There are some enzymes that have relatively low specificities (*bond specificity*), e.g. certain peptidases, phosphatases, and esterases, which will utilize a wide range of substrates provided they contain the required chemical bond, i.e. peptide, phosphate ester, and carboxylate ester, respectively, in these three examples. Low specificity is more commonly encountered with degradative enzymes but is only very rarely observed with biosynthetic enzymes. The role of the former may be that of digestion, where wider specificity would be more economical. An intermediate set of enzymes show *group specificity*, e.g. hexokinase. This enzyme will catalyse the phosphorylation of a variety of sugars provided they are aldohexoses. However, many enzymes show *absolute* or *near-absolute specificity*, in which they will only catalyse at an appreciable rate the reaction with a single substrate (or a single pair of substrates in a bimolecular reaction), e.g. urease will only catalyse the reaction with urea, or with very similar analogues at a very much lower rate. The quantitative definition of specificity in kinetic terms is described in Chapter 4, Section 4.3.1.3.

The terms *group specificity* and *absolute specificity* can be readily appreciated in relation to low-M_r substrates, but when considering macromolecular substrates the position may be somewhat different in the sense that the active site of the enzyme can only interact with a part of the macromolecule. This is simply due to the relative dimensions of the active site and the macromolecule. A group of enzymes that has been studied extensively in the past decade is the restriction endonucleases. These enzymes generally recognize a sequence of four to six base pairs in DNA and then cleave the phosphodiester links in both strands but not necessarily in opposite positions. There are now known at least 400 of these enzymes differing in specificity.[17] In a sense, each enzyme shows absolute specificity for the region of the substrate that is in contact with the active site, although it can act on any DNA molecule or fragment that contains the appropriate sequence.

Another distinct feature of many enzyme-catalysed reactions is their stereospecificity; this is well illustrated in the case of NAD^+- and $NADP^+$-requiring dehydrogenases. It has been demonstrated by use of suitably labelled substrates that dehydrogenases catalyse the transfer of hydrogen from the substrate on to a particular side of the nicotinamide ring; these are designated A-side and B-side dehydrogenases (Fig. 1.2). In addition, almost all dehydrogenases act on either NAD^+ or $NADP^+$. The basis of these specificities is clear in the case of those dehydrogenases whose three-dimensional structures are known, e.g. liver alcohol dehydrogenase, lactate dehydrogenase, and glyceraldehyde-3-phosphate dehydrogenase[19] and is discussed further in Chapter 5, Section 5.5.4.3.